

## Examples of Biospecimen Research: Intra-operative Variables and Cancer Tissue

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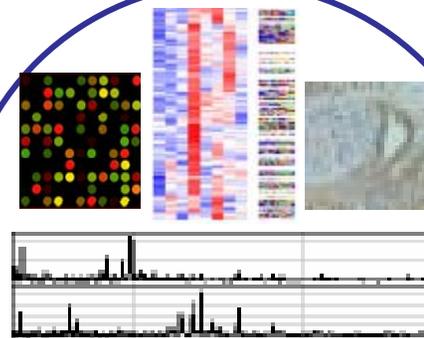
## A biotech company integrating clinic, biobanking and research



### Indivumed personnel in Clinical Centers



### High Quality Biobanking



### Research and Data Analysis

### Diagnostics & Therapeutics

Target  
identification

Target  
validation

Biomarker  
development

Predictive  
preclinical drug tests

Support of  
clinical trials

## Clinical Centers and tumor entities



Israelitic Hospital Hamburg General Surgery
Israelitic Hospital Hamburg Gastroenterology
Diakonie-Hospital Alten Eichen General Surgery
Diakonie-Hospital Elim-Hospital Gynecology
Diakonie-Hospital Alten Eichen Plastic Surgery
General Hospital Hamburg-Harburg Lung Center/Thorax Surgery
University Hospital Hamburg- Eppendorf / Hepatobiliary Surgery
University Hospital Hamburg- Eppendorf / Urology
University Hospital Göttingen General Surgery



### Indivumed personell:

- Assure consent
- Collection of blood/urine
- Documentation of surgery
- Documentation of anesthesia
- Collection of tissue
- Processing biospecimen
- Quality management
- Documentation hospital stay
- Disease follow-up
- Follow-up biospecimen

## The Indivumed-Standard (I): Defined, controlled and rapid biospecimen collection



### *Tissue:*

**Set of Normal+Tu-center+Tu-periphery**

**Ischemia time < 12 min**

**Blocks of similar size**

**Simultaneous N<sub>2</sub>-freezing of tissues**

**Formalin fixation of 16 hours**

### *Fluids:*

**Instant cooling of blood/urine (4 °C)**

**Serum/plasma/MNC processing < 4 hours**

**Urine supernatant/sediment < 4 hours**



# The Indivumed-Standard (II): Complete, prospectively collected and verified clinical data

## Biospecimen: Collection/processing



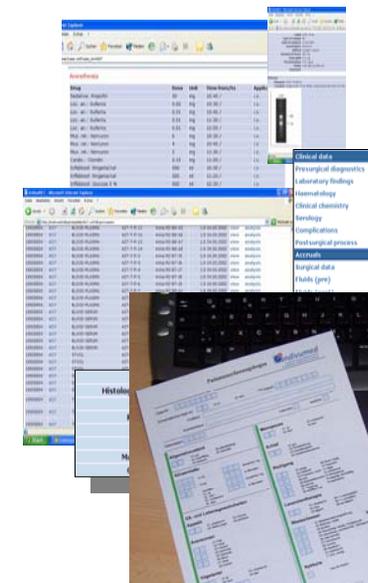
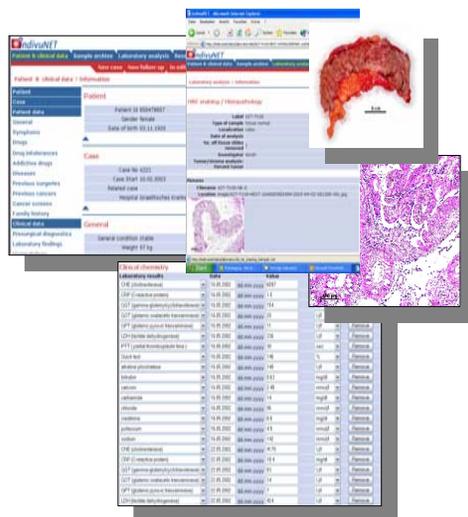
## Surgery: Procedure/drugs



## Patient: History/hospital

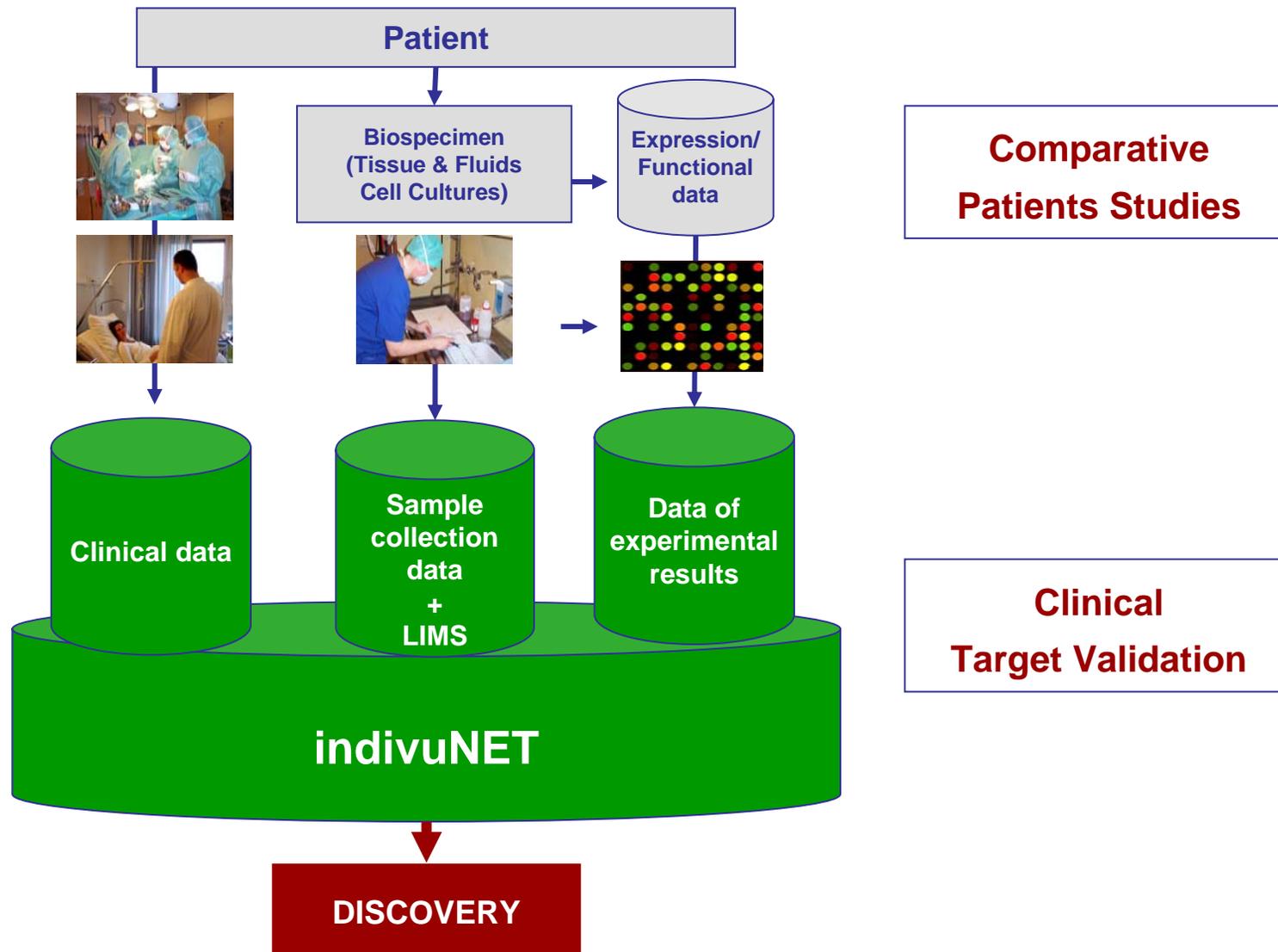


## Patient: Therapy/outcome

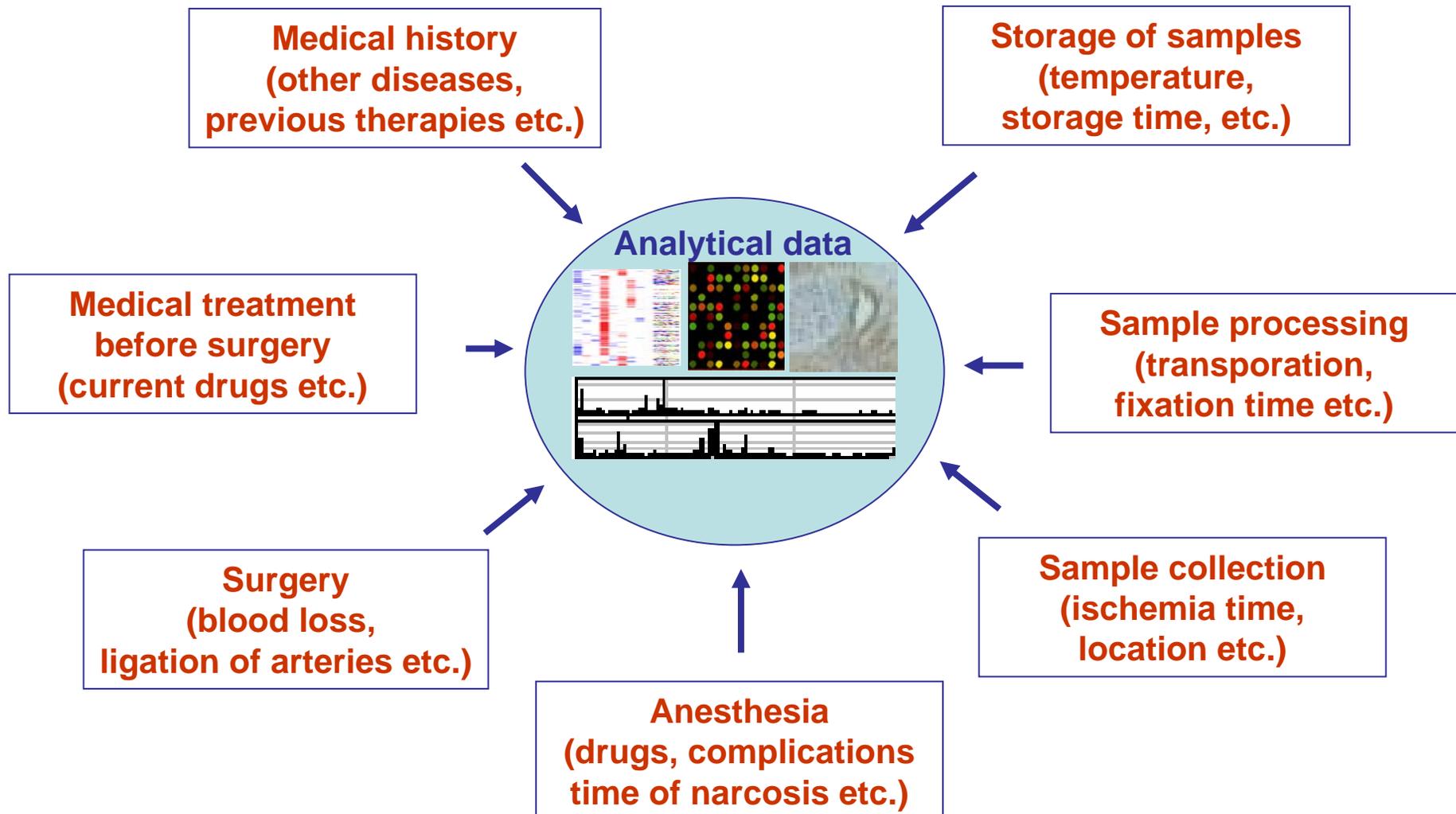


- ✓ Standardized documentation
- ✓ Complete data (300 data points)
- ✓ Prospective collection
- ✓ Web-accessible data base
- ✓ Compatible data format (Oracle)

## Integrated Analytical Platform



## Overview of exogenous factors affecting molecular data



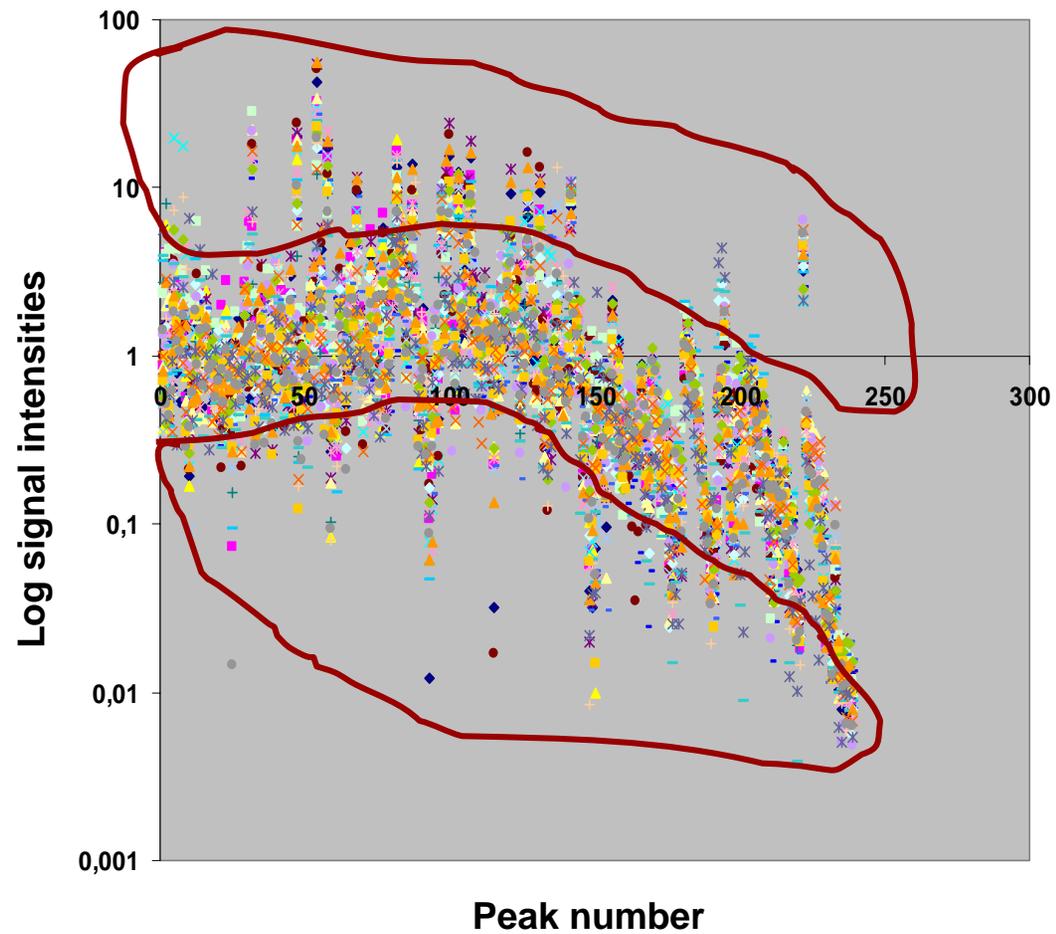
## Critical variables for science guided biobanking



**Statistical analysis of protein  
expression data in normal colon  
tissue**



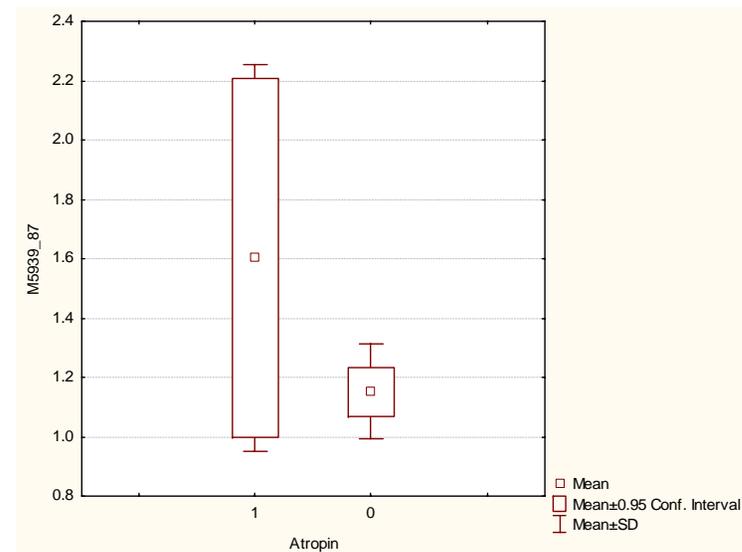
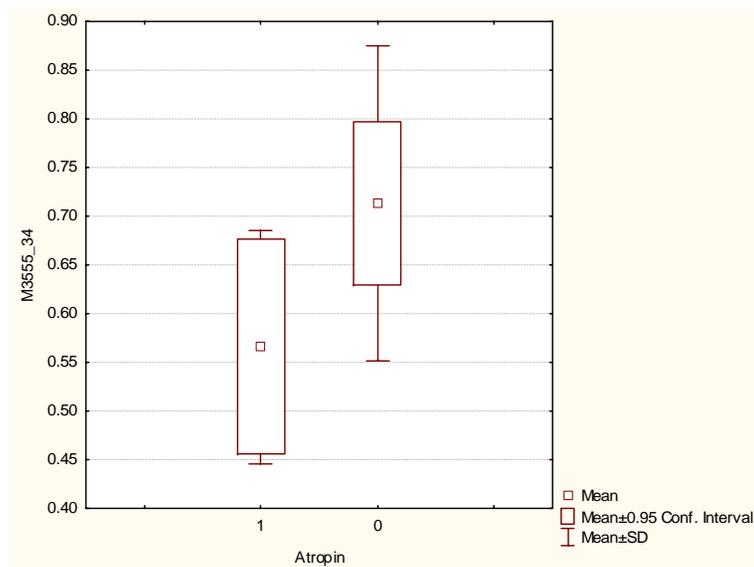
## SELDI-TOF-MS analysis of 24 normal colon cancer (CM-10 chip) Total number of detectable peaks n=238



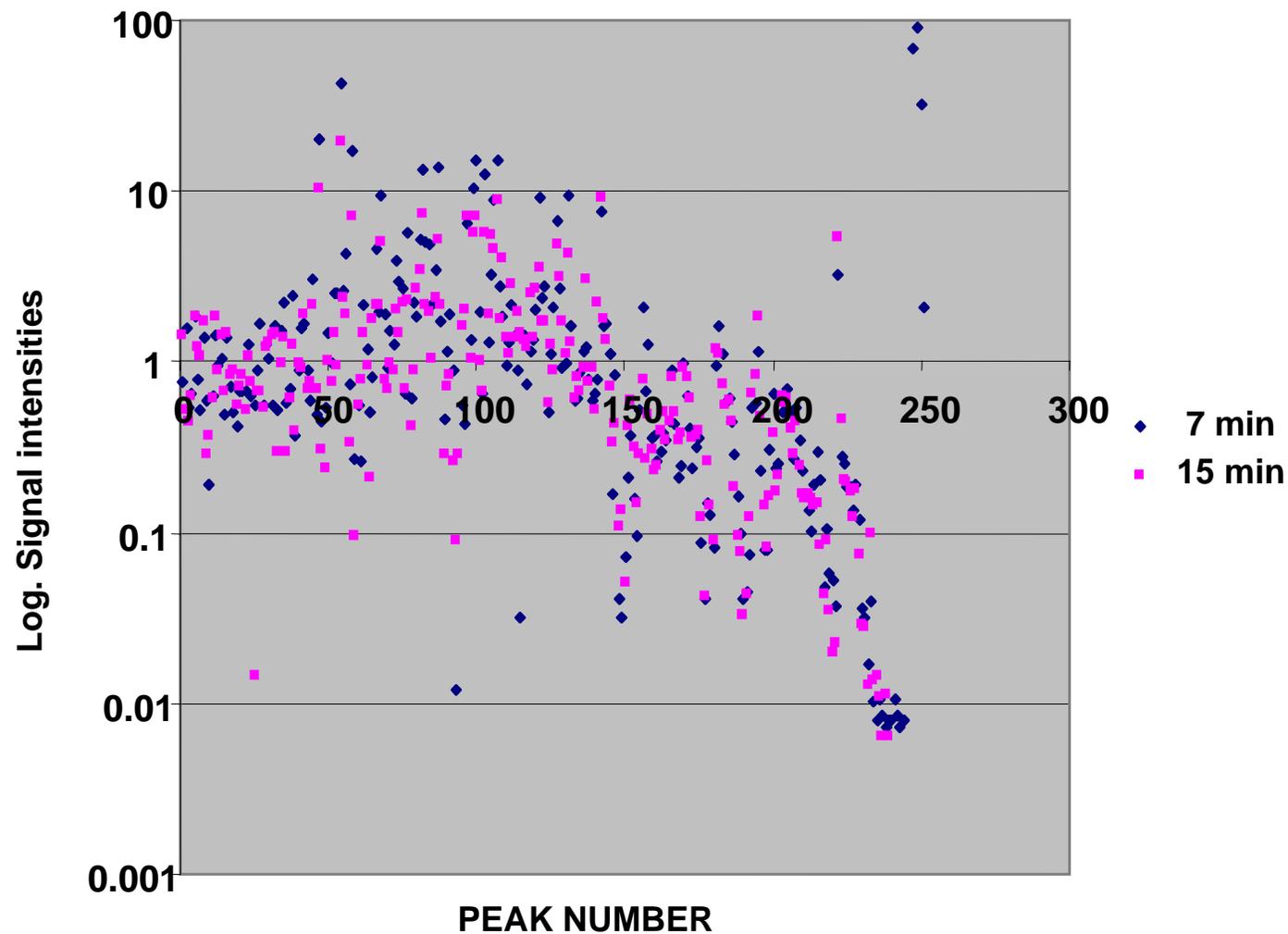
## Impact of atropin on protein pattern:

4 peaks different from 238 (1.7 %) Atropin treated vs. non treated

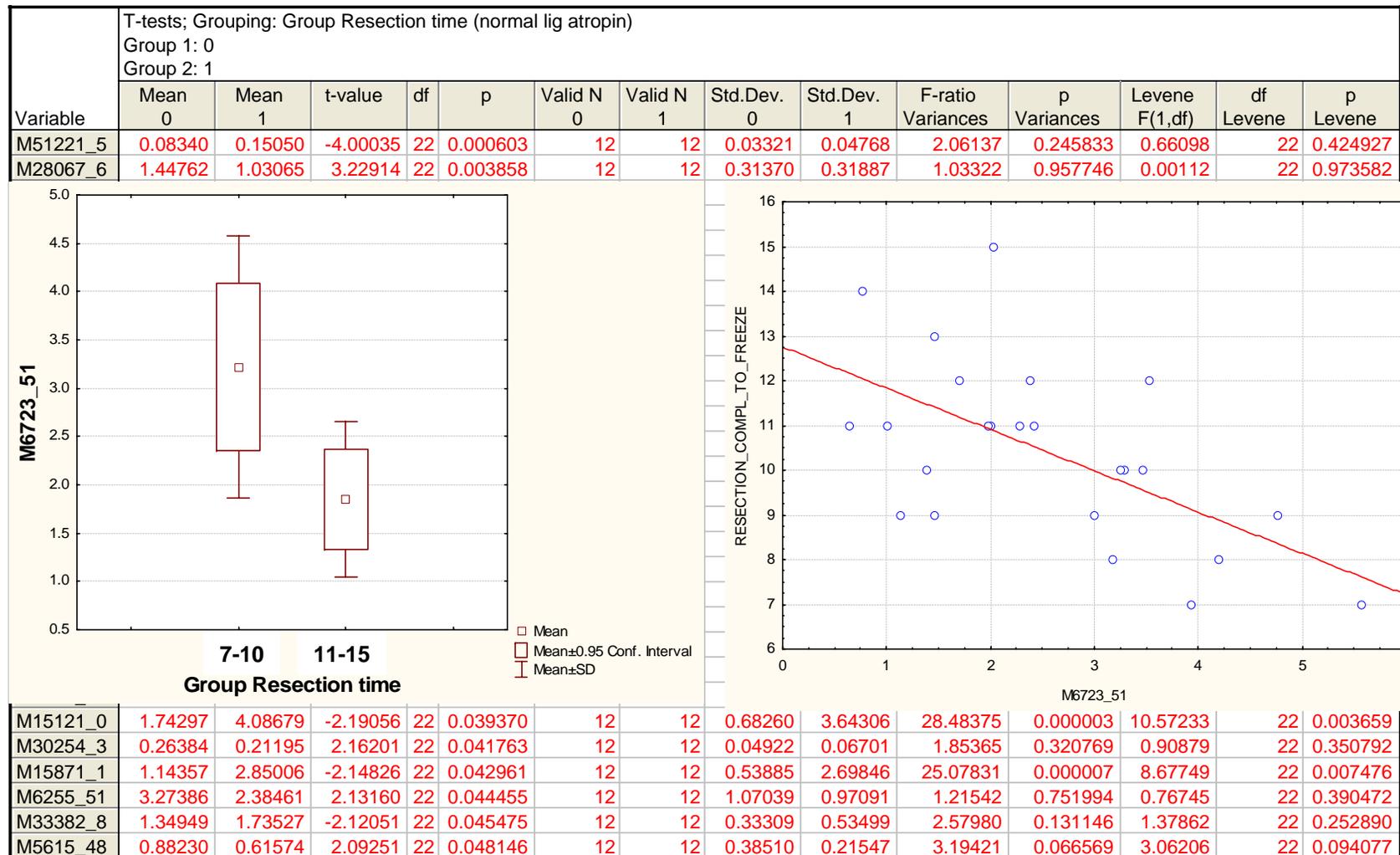
T-tests; Grouping: Atropin (normal lig atropin.sta)									
Group 1: 0									
Group 2: 1									
Variable	Mean 0	Mean 1	t-value	df	p	Valid N 0	Valid N 1	Std.Dev. 0	Std.Dev. 1
M5939_87	1.15213	1.60276	-2.73669	22	0.012043	17	7	0.15888	0.65240
M3772_14	0.63586	1.05263	-2.34306	22	0.028574	17	7	0.25252	0.63653
M6723_51	2.17426	3.41282	-2.31784	22	0.030148	17	7	0.97299	1.63301
M3555_34	0.71346	0.56601	2.16344	22	0.041640	17	7	0.16216	0.11972



## Variation of tissue ischemia time between 7-15 min



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29 peaks (12.1 %) out of 238 are different

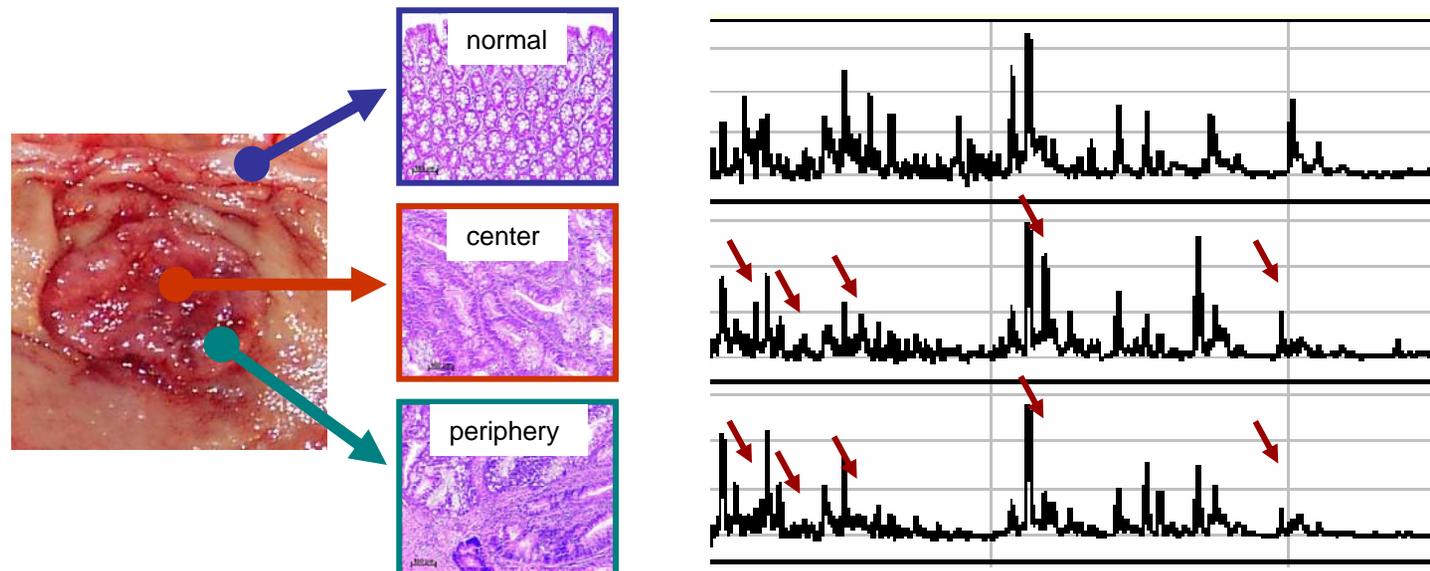
## Critical variables for science guided biobanking



**Controlled study analyzing:**  
-different tumor areas  
-impact of ischemia and

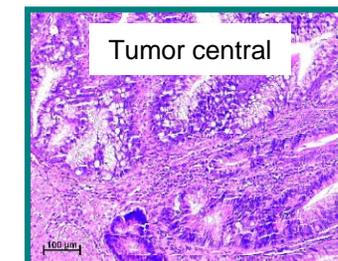
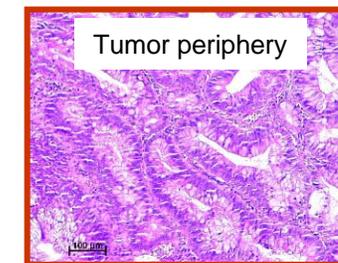
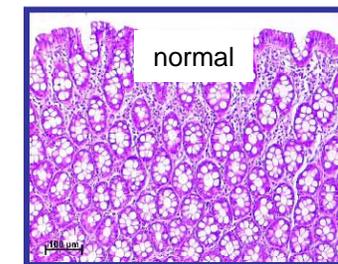
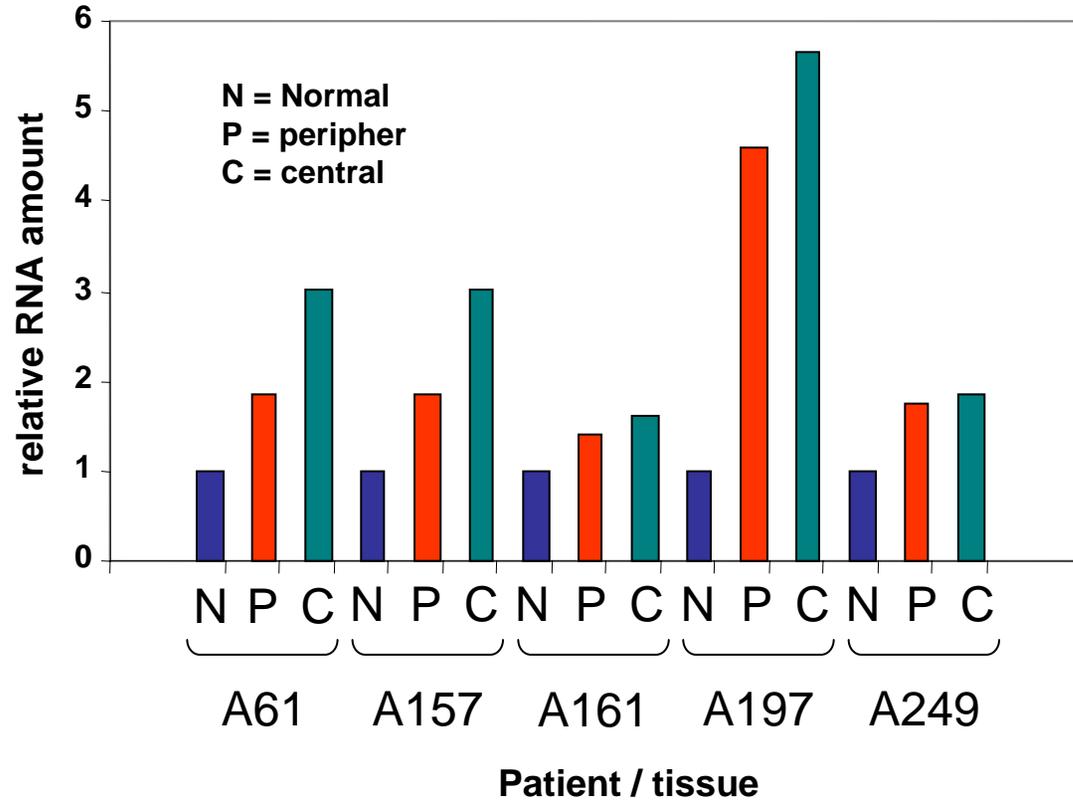


## Localization of tumor biopsy affects molecular pattern (SELDI-TOF-MS analysis)



**Approx. 40% of proteins are differentially expressed between  
peripheral and central tumor regions**

## Expression of VEGF in different tissues: normal - periphery - central (real-time RT-PCR)

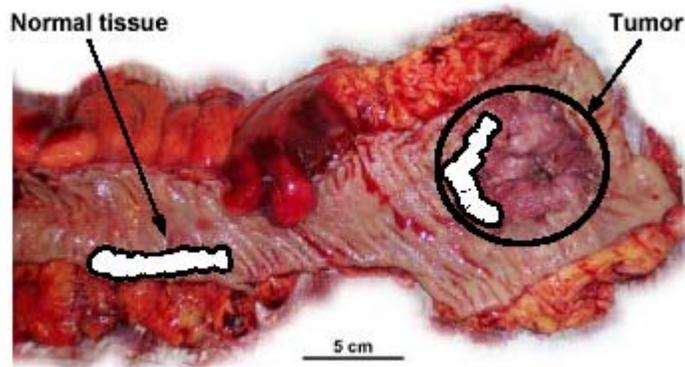


## Impact of cold ischemia: controlled tissue collection

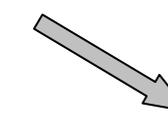
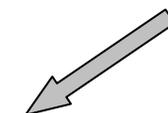
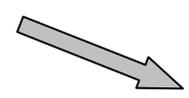


**Surgical removal  
of rectum**

**Collection of normal and cancer tissue**

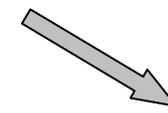


**Control of warm ischemia**



**Tissue collection following resection:  
Snap frozen in liquid N2**

- after 5 min
- 8 min
- 10 min
- 12 min
- 15 min
- 20 min
- 25 min
- 30 min

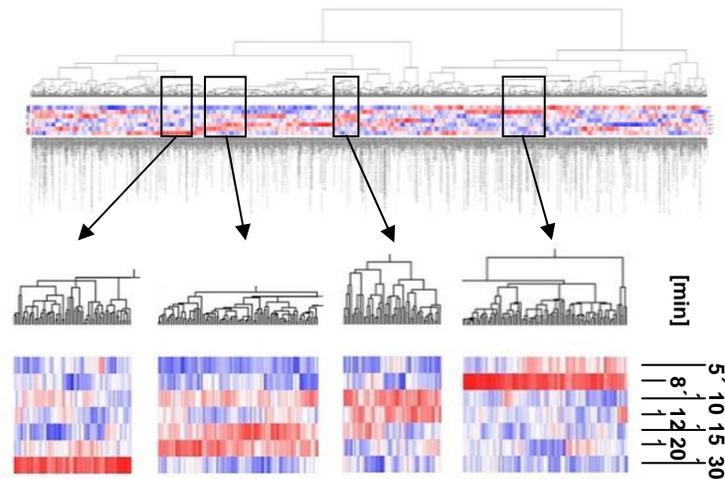


**Analysis:**

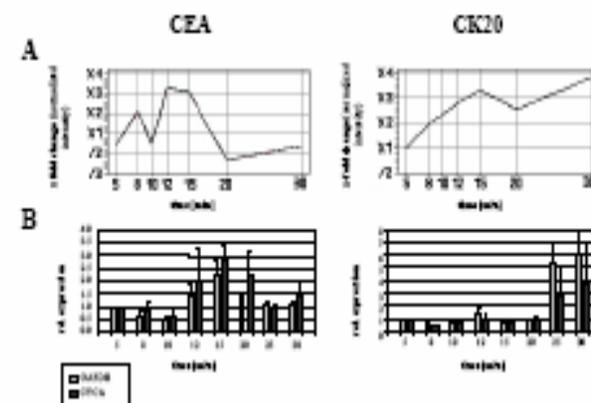
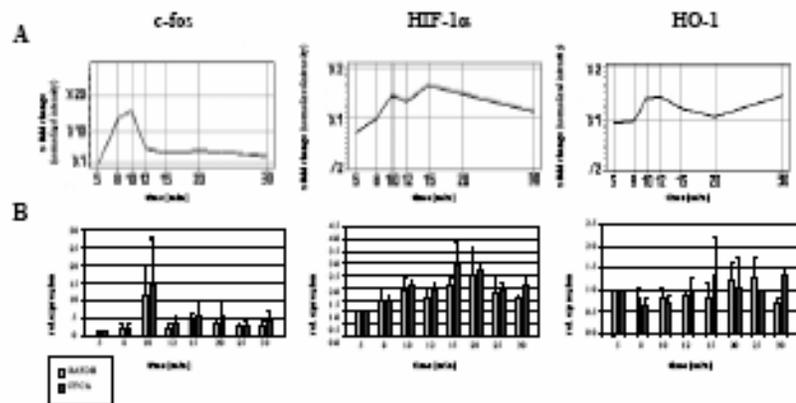
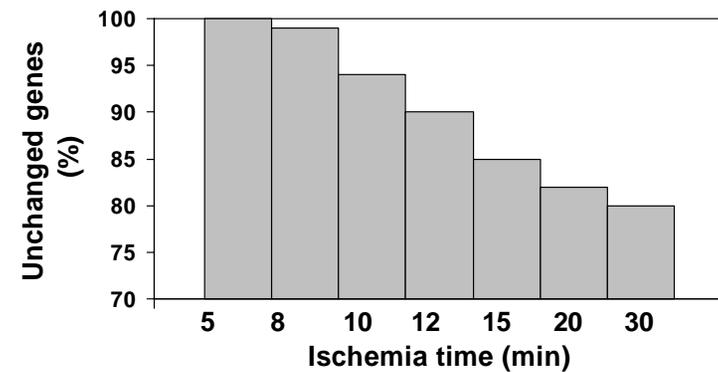
- Affymetrix
- real-time RT-PCR
- SELDI-TOF-MS



## Tissue ischemia and gene expression profiling (Affymetrix cDNA microarray and real-time RT-PCR)

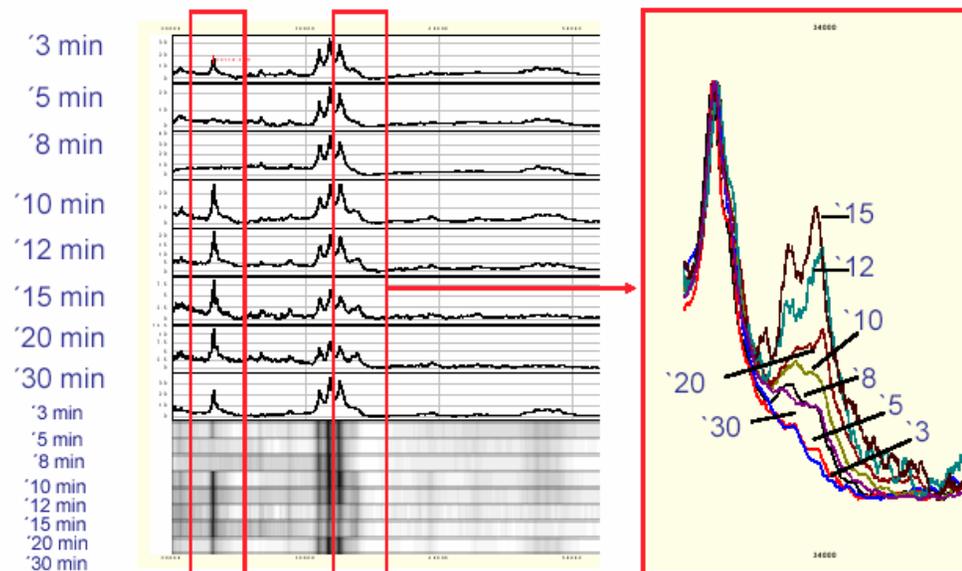
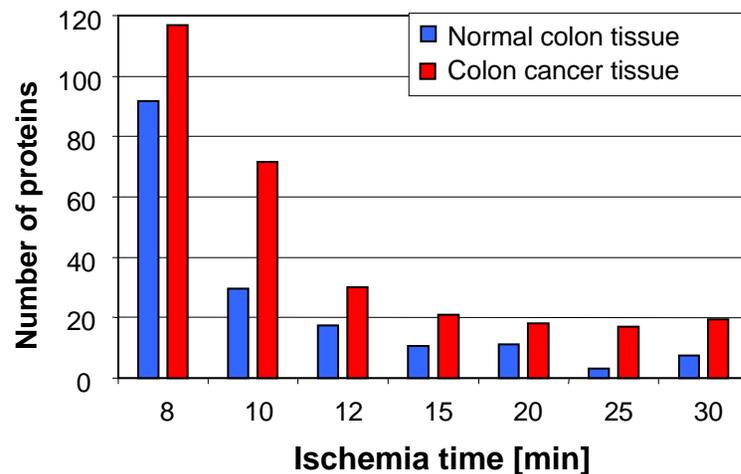


Time course of gene expression



Following tumor resection ~ 20-25% of genes are differentially expressed within the first 30 minutes !

## Tissue ischemia time and protein expression in colon tissue (SELDI-TOF-MS analysis)



**Following tumor resection ~ 25-30% of proteins are differentially expressed within the first 30 minutes !**

## Tissue requirement in gene and protein expression studies for target discovery and validation

- > 20 min ischemia: 20-30% of artifacts in RNA/protein profiling
- < 15 min ischemia time: reduction of ischemia-related artifacts by 50%
- < 10 min ischemia time: reduction of ischemia related artifact by 80%

**Control of ischemia time during tissue collection is essential.**

**Any minute affects research productivity!**

## Summary

Molecular tissue data are related to numerous pre- and intrasurgical variables such as:

- Drugs before and during surgery
- Tumor area
- Tissue ischemia time
- Others

**Goal of science-guided biobanking:**

**Biospecimen should reflect molecular reality of patients!**



**Bridging the gap between preclinical models and patients**

